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EVALUATION OF SPERMATOLOGICAL PARAMETERS AND FREE AMINO ACIDS COMPOSITION OF BLACK BALADI, NEW ZEALAND WHITE AND V-LINE RABBITS BUCK UNDER WINTER EGYPTIAN CONDITION

By

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Summary: *Twenty one mature male rabbits of Black Baladi (BB) as a local breed and both of New Zealand white (NZW) and V-Line (VL) rabbit bucks as an exogenous breed (seven rabbits from each breed) were used to study their semen quality, enzymatic properties and free amino acids content of seminal plasma under Egyptian condition. Ejaculate volume (EV ml), sperm motility(SM), sperm concentration (SC), total sperm output (TSO), total motile sperm (TMS), total functional sperm fraction (TFSF) and normal sperm showed significantly higher in NZW and VL compared with BB rabbits. Semen initial hydrogen ion concentration (pH), reaction time (RT) and dead sperm values were significantly increased in the BB than the NZW and VL rabbits. NZW and VL rabbits recorded the highest means in seminal plasma total protein and albumin concentration these were significantly increased compared with BB rabbits buck. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in NZW and VL were significantly decreased than the BB rabbits, while, acid phosphatase (AcP) and alkaline phosphatase (AlP) activities were significantly increased only in VL compared with NZW and BB rabbits. Data of free amino acids revealed that total amount of free amino acids in the seminal plasma was increased in VL and NZW than BB breed. Seminal plasma of BB breed contained 17 free amino acids while NZW and VL contained 16 free amino acids and this difference between BB and other two rabbit breeds due to absence of Serine from the last two breeds. The largest portion of free amino acids was Cystein in seminal fluid of the three breeds.*

INTRODUCTION

Several methods have been used for routine analysis of mammalian semen, each describing specific quality criteria of the sperm. Semen analysis is the initial step in the evaluation of male reproductive performance (Mohamed et al., 1986). Standard semen analysis including sperm concentration, motility and morphology is widely used as a fundamental indicator of male fertility (Liu and Baker, 1992).

Most recently, Saacke et al., (2000) in cattle and Castellini and Lattaioli (1999) in rabbits found that sperm number and number of motile sperms are positively associated with both fertilization rate and embryonic quality. As well as, Yousef et al., (2001) reported that in rabbits it is possible to select bucks as sires for breeding programs depending on number of motile sperm per ejaculate, but not percentage of progressive motility per sires.

Seminal plasma free amino acids are produced in the testes, epididymis and sex accessory glands and also by action of endogenous proteolytic enzymes on seminal proteins after ejaculation (Roussel and Stallcup, 1967). Hopwood and Gassner (1962) noted a positive correlation between free amino acid concentration and the fertilizing capacity of bull semen. Ibrahim and Boldizsár (1981) found that the average fertility score of bulls containing elevated free amino acids concentration was slightly higher than the bulls of low amino acids level.

Al-Hakim et al. (1970) verified a linear correlation between glutamic and aspartic acids and fertility of Holstein bulls. On the other hand, no direct relationship between seminal plasma free amino acids and fertility has been observed, except for glutamic acid that, together with aspartic acid, serine, alanine and glycine, constitute the largest portion of free amino acids in this fluid (Roussel and Stallcup, 1967).

The purpose of this study was to identify affiliation in semen quality and seminal plasma free amino acid composition in local breed rabbit (Balade) and two foreign breeds which acclimated under local condition (New-Zealand and V-Line rabbits).

MATERIAL AND METHODS

1- Design and management:

This study was carried out at El-Sabahia Poultry Research station, Animal production Research Institute, Agriculture Research Center and

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Twenty one mature male rabbits of black Baladi, New Zealand white and V-Line rabbits buck (seven rabbits from each breed) 7-months old (with average initial weight 3.254 kg) were used during winter time. The rabbits were individually housed in metal cages, feed and water were provided *ad libtum*. The composition of the ingredients of pelleted concentrate feed (% on a dry matter basis) are shown in Table (1).

2- Semen physical characteristics:

The experimental period was continuing for 8 weeks. Semen samples were individually collected weekly using an artificial vagina. Ejaculate volume was recorded nearest 0.1 ml (using a graduated collection tube) after removal of the gel mass. A weak eosin solution (Smith and Mayer, 1955) was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide. Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration. The percentages of motile sperm were estimated by visual examination using a phase-contrast microscope with heated stage. Total number of motile sperm (TMS) was calculated by multiplying percentage of motile sperm and total sperm outputs. Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility by normal morphology sperm (Correa and Zavos, 1996). Assessment of live, dead, and abnormal sperm were performed using an eosin-nigrosine blue staining mixture (Blom, 1950). Initial hydrogen ion concentration (pH) of semen samples was determined just after collection using a pH cooperative paper ranging from 0 to 14 with one grade. Reaction time(second) was determined as the moment of subjecting a doe to the buck.

3- Semen chemical characteristics:

Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C, and was stored at -20 °C until later analysis. By using commercial kits seminal plasma samples were analyzed for total protein (TP) by the Biuret method according to Henry et al. (1974). Albumin (A) concentration was determined by the method of Doumas et al. (1977). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). For assaying acid phosphatase (AcP) activity, the method of Moss, (1984) was used. Alkaline phosphatase (AlP) activity was measured according to methods of Principato et al., (1985).

Free amino acids in seminal plasma were extracted as described by Hamilton(1962) and the individual free amino acids were measured using a method which described by Spackman et al. (1958) using amino acid analyzer system (model: SYKAM S 7130).

Data were analyzed as a completely randomized design (Steel and Torrie, 1980) using the General Linear Model procedures of SAS (1986). Significance of the effects was tested at levels $p<0.05$ and $p<0.01$ with the appropriate F statistic. Duncan's multiple range test was used to detect any significant differences among the experimental means (Duncan, 1955).

RESULTS AND DISCUSSIONS

1-Semen physical characteristics:

Results in Table (1) are presented the data of ejaculate volume (EV), sperm motility (%), initial hydrogen ion concentration (pH) and reaction time (RT) for three rabbit breeds (BB, NZW and VL respectively). The data revealed that NZW had significantly higher semen ejaculate volume than both BB and VL rabbits, while, no significant differences between VL and BB rabbits was found. BB rabbit showed a significantly decreased in sperm motility compared with VL which had significantly lower motility. Seminal plasma pH values were significantly higher in BB followed by VL and NZW rabbits. The results indicated that there was a relationship between the decreased pH value and increased sperm motility. This decrease in pH value of NZW and VL rabbits may be resulted from the increased sperm metabolite activity. Reaction time was significantly shorter in NZW rabbits compared to BB rabbits.

Data in Table (2) showed that VL rabbits recorded highly significant sperm concentration than both NZW and BB rabbits, BB was the most declines. In spite of, VL rabbits had the higher sperm concentration values, but the total sperm output (TSO) was significantly higher in NZW rabbits that may be due to the increase of semen ejaculate volume. The percent decrease in dead sperm was significantly lower in both NZW and VL compared with BB rabbits (Table 2). Regarding to the percentage of normal sperm can be mentioned that VL rabbits showed significantly higher in this point compared with the NZW and BB breed, this higher was reflected to decreasing the percentage of abnormal sperm. In contrast, BB breed showed significantly higher in the percentage of abnormal sperm and significantly lower in percentage of the normal sperm. In addition, decreasing of TMS and TFSF in BB breed may be due to increasing the percentage of dead and abnormal sperm compared with the VL and NZW breed. The data revealed also that, in spit of VL breed had significantly higher sperm concentration

and percentage of live and normal sperm, the TMS and TNSF were significantly higher in NZW than VL and this increasing might be due to the increasing at the ejaculated volume of NZW (Table 2).

The present results were found to agreement with that of Mohamed et al., (1986) who reported that the percentage of motile spermatozoa and the quality of their forward progression have appeared to be the best prediction of humane male fertility potential. Furthermore, sperm motility was found to be one of the most important criteria of semen quality and a determinant in the success of fertilization (Ijaz et al., 1994). In addition to Castellini and Lattaioli (1999) with rabbits, found that sperm number and number of motile sperms was positively associated with both fertilization rate and embryonic quality. In conclusion with rabbits it is possible to select bucks as sires for breeding programs depending on number of motile sperm per ejaculate, but not percentage of progressive motility per sires (Yousef et al., 2001)

2-Semen chemical characteristics:

Semen chemical characteristics of three studied rabbits breeds were presented in Table (3). Data showed that, seminal plasma total protein (TP) was significantly higher in VL compared with NZW or BB breeds. As well, seminal plasma albumin (Alb) was taken the same trend of TP and the differences were statically significant between the three breeds. Also, it can be observed that there was a positive relationship between the increase percentage of sperm motility, normal sperm and total motile normal sperm with total protein and albumin levels in seminal plasma. According to Kamel (2005) seminal plasma total protein and albumin levels were increased with the increasing seminal quality in rabbits Additionally, many studies have shown also that low content seminal plasma protein is associated with poor semen quality (Verma et al., 1985; Dhami and Kodagali, 1989, Taha et al., 2000; and Osama and Amany, 2006) whereas, seminal plasma protein make up the amphoteric property of seminal plasma low protein content in seminal plasma reduce its buffering capacity and in turn semen quality (Dhami et al., 1994).

Local BB breed showed significantly increased in ALT and AST enzymes activities compared with the other two rabbit breeds (Table 3). According to Pursel et al. (1968), Yousef et al. (2003) and Yousef and Zeitoun (1998) the increases in ALT and AST activities was concurrent with the decline in semen quality. There was a negative correlation between increased ALT and AST activities and ejaculate volume, sperm concentration, total sperm output, sperm motility index, total motility index,

total motile sperm. They reported that the activities of these enzymes could be used as an indicator of sperm integrity

AcP and AlP enzymes activities were significantly increased in seminal plasma of VL than NZW and BB breeds (Table 3). Previous studies showed that rabbit's seminal plasma contained a number of enzymes, these enzymes play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism, in fertilization process and in the maintenance of constant osmotic pressure during preservation (Yousef et al., 2003 and Dhami and Kodagali, 1987). Phosphatases in semen play an important role in phosphorylation processes in sperm metabolism (Dhami et al., 1994). In the results of this study that may be explain the differences observed in the semen quality of the three breeds, that agree with the results of Kamel (2005) in rabbits which have higher seminal quality index the AcP activity was higher in their seminal plasma.

3-Seminal plasma free amino acid composition:

Data of free amino acids analyzer represented in Table (4) and Figures (1-3). Total amount of free amino acids of seminal plasma was higher in VL followed by NZW while BB breed had the lowest quantity than other two breeds (128.04, 73.02 and 57.165 mg/100ml, respectively). On the other side, seminal plasma of BB breed contained 17 free amino acids while NZW and VL contained 16 free amino acids and this difference between BB and other two rabbit breeds due to absence of Serine from the last two breeds. Cystein constitutes the largest portion of free amino acids in seminal fluid of all breeds (Table 4). VL breed had the highest level (47.6mg/100ml seminal fluid) then NZW (36.4mg/100ml) while, BB (34.5mg/100ml). On the other hand, BB seminal fluid had the highest percentage from cystein then NZW and VL which was the lower (60.325, 49.842 and 37.2 %) from total free amino acids in BB, NZW and VL seminal fluid respectively (Figure 3). Also, free amino acids results revealed that, in BB seminal fluid, the free amino acids with the highest concentrations were Cystein, Glycine, Phenylalanine and Arginine, which represent about 77.10% from total free amino acids. Also, in NZW there were seven amino acids constitute approximately 87.386 % from total free amino acids seminal fluid (Cystein, Glycine, Phenylalanine, Arginine, Lysine, Glutamic acid and Histidine). VL seminal fluid however, had highest amount of Arginine concentration compared to BB or NZW breeds, also, BB breed had lower Lysine and Histidine content compared with the other two breeds.

Table (4): Overall means of seminal plasma total protein (TP), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (AcP) and Alkaline phosphatase (AlP) in Black Baladi (BB), New Zealand white (NZW) and V line (VL) rabbits during winter season (Mean±SE).

Items	BB	NZW	VL
TP (g/dl)	5.81 ± 0.12^{B}	5.91 ± 0.05^{B}	6.25 ± 0.08^{A}
Alb (g/dl)	2.80 ± 0.05^{C}	2.95 ± 0.07^{B}	3.04 ± 0.04^{A}
ALT (U/l)	14.78 ± 0.46^{A}	12.45 ± 0.23^{B}	12.41 ± 0.38^{B}
AST (U/l)	12.51 ± 0.30^{A}	11.82 ± 0.26^{AB}	11.58 ± 0.49^{B}
AcP (U/l)	31.42 ± 0.30^{B}	31.50 ± 0.30^{B}	32.72 ± 0.31^{A}
AlP (U/l)	51.02 ± 1.19^{B}	50.91 ± 0.96^{B}	52.00 ± 0.97^{A}

^{ABC} means the same row have the different superscript are significantly different at ($p\leq 0.05$).

Table (5): Free amino acids contents (mg/100ml) in seminal plasma of Black Baladi (BB), New Zealand white (NZW) and V line (VL) rabbits during winter season.

Free Amino Acid	BB	NZW	VL
Aspartic acid	0.7	1.12	0.96
Threonine	1.235	1.845	1.445
Serine	1.225		
Glutamic Acid	1.225	3.31	4.47
Proline	1.165	2.21	1.375
Glycine	5.03	5.145	10.17
Alanine	1.1	0.91	1.1
Cystein	34.485	36.395	47.63
Valine	1.77	0.385	2.95
Methionine	0.155	1.545	1.555
Isoleucine	1.08	1.47	3.18
Leucine	0.285	0.27	0.39
Tyrosine	1.175	1.185	3.15
Phenylalanine	2.395	3.17	12.17
Histidine	0.91	1.935	5.24
Lysine	1.065	7.23	10.12
Arginine	2.165	4.895	22.09
Total	**57.165**	**73.02**	**128.04**

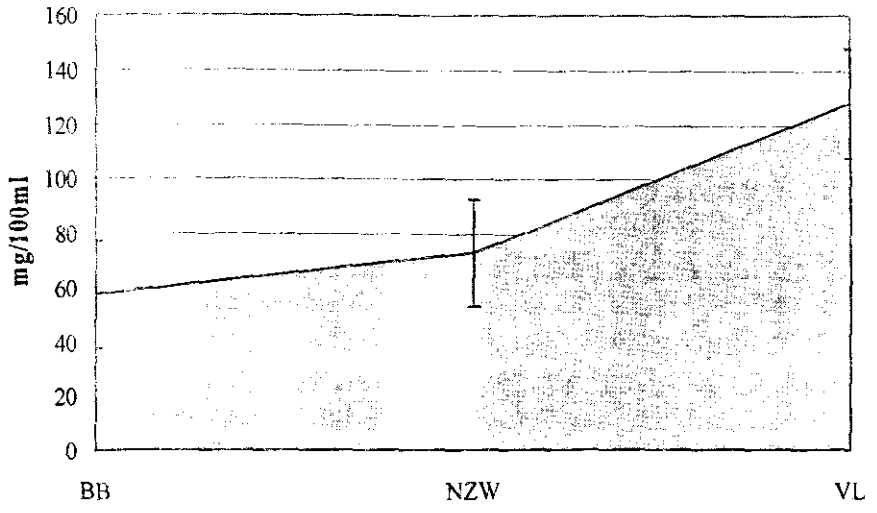

Figure 1: Total amino acid in seminal plasma of Black Baladi (BB), New Zealand white (NZW) and V-Line (VL) rabbit bucks.

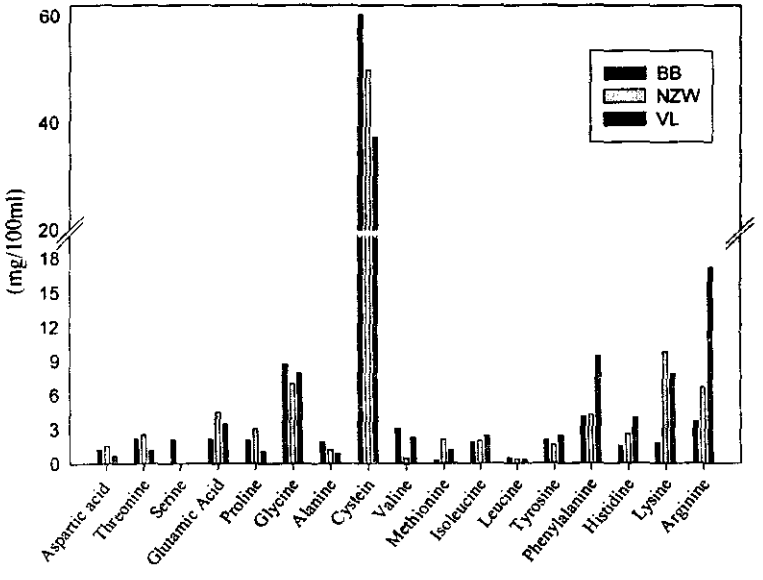

Figure 2: Free amino acid concentration in seminal plasma of Black baladi (BB), New Zealand white (NZW) and V-Line (VL) rabbits bucks.

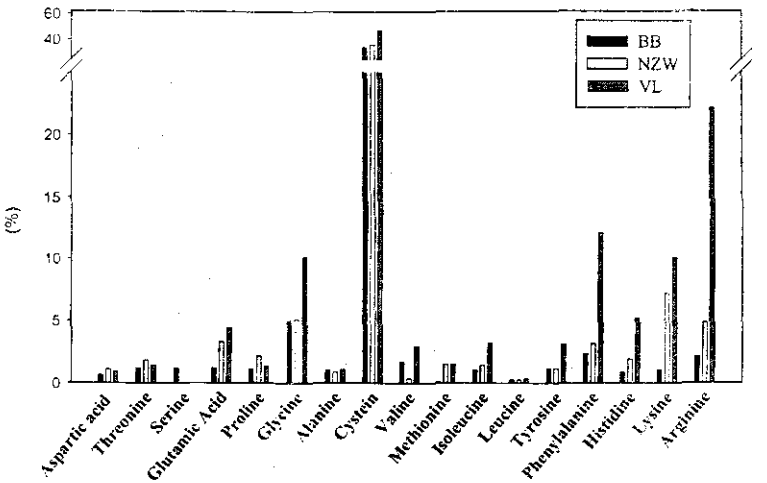

Figure 3: Free amino acid in seminal plasma as a percentage from total amount of free amino acids of Black Baladi (BB), New Zealand white (NZW) and V-Line (VL) rabbit bucks.

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الملخص العربي

جودة السائل المنوي والمحتوي من الأحماض الامينية الحرة لكل من الأرانب البلدي الأسود والنيوزيلندي والفيلايين تحت ظروف الشتاء في البيئة المصرية

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مدينة مبارك للابحاث العلمية والتكنولوجي**

اجريت هذه الدراسة بمحطة بحوث الدواجن بالصبحية التابعة لمركز البحوث الزراعية بالاشتراك مع مدينة مبارك للابحاث العلمية. هدفت الدراسة الي البحث في اختلافات خصائص السائل المنوي ومحتواه من الاحماض الامينية الحرة في ذكور الارانب من سلالات البلدي الاسود كسلالة محلية وكل من السلالتين النيوزيلندي والفيلايين كسلالات اجنبية تربي تحت ظروف فصل الشتاء المصرية. تم استخدام 21 من ذكور السلالات الثلاثة (7 من كل سلالة) استخدمت لدراسة صفات السائل المنوي وتركيز الانزيمات ومحتوي السائل المنوي من الاحماض الامينية الحرة لكل سلالة علي حدة. اظهرت الدراسة ان حجم القذفة، الحركة الكلية للحيوانات المنوية ،تركيز الحيوانات المنوية، العدد الكلى للحيوانات المنوية فى القذفة، عدد الحيوانات المنوية المتحركة لكل قذفة ، عدد الحيونات المنوية الطبيعية (الغير شاذة)، وعدد الحيونات المنوية الطبيعية المتحركة لكل قذفة كانت اعلي معنويا في السلالات النيوزيلندي والفيلايين مقارنا بالسلالة البلدي. درجة الحموضة و الرغبة الجنسية وعدد الحيوانات المنوية المشوهه في الأرانب البلدي كانت اعلي معنويا من السلالات النيوزيلندي والفيلايين. اظهرت محتوي السائل المنوي من البروتين الكلي والالبيومين اعلي نسبة معنوية في السلالات النيوزيلندي والفيلايين مقارنا بسلالة البلدي. نشاط الانزيمات اسبرتيت ترنزامينيز والانيين ترنزامينيز كان الاقل معنويا في السلالات النيوزيلندي والفيلايين بينما كا نشاط انزيم الفوسفاتيز القاعدي والحامض فوسفاتيز اعلي معنويا مقارنا بالبلدي الاسود. محتوي الاحماض الامينية الحرة في البلازما المنوية كان اعلي في السلالات النيوزيلندي والفيلايين عن البلدي الاسود. بينما كان عدد الاحماض الامينية الحرة في ارانب البلدي 17 حمض اميني في حين كان هذا العدد 16 حمض اميني في السلالات النيوزيلندي والفيلايين وكان هذا الاختلاف راجع الي غياب الحمض الاميني السيرين. اكبر نسبة من الاحماض الامينية كان من نصيب الحمض الاميني السيستيين في الثلاث سلالات.